# A new case of ALG8 deficiency (CDG Ih)

Vesela, K ; Honzik, T ; Hansikova, H ; Haeuptle, M A ; Semberova, J ; Stranak, Z ; Hennet, T ; Zeman, J

Abstract: Congenital disorders of glycosylation (CDG) represent an expanding group of inherited diseases. One of them, ALG8 deficiency (CDG Ih), leads to protein N-glycosylation defects caused by malfunction of glucosyltransferase 2 (Dol-P-Glc:Glc1-Man(9)-GlcNAc(2)-P-P-Dol glucosyltransferase) resulting in inefficient addition of the second glucose residue onto lipid-linked oligosaccharides. So far, only five patients have been described with ALG8 deficiency. We present a new patient with neonatal onset. The girl was born at the 29th week of gestation complicated by oligohydramnios. Although the early postnatal adaptation was uneventful (Apgar score 8 and 9 at 5 and 10 min), generalized oedema, multifocal myoclonic seizures, and bleeding due to combined coagulopathy were present from the first day. Diarrhoea progressing to protein-losing enteropathy with ascites and pericardial effusion developed in the third week of life. Pharmacoresistant seizures and cortical, cerebellar and optic nerve atrophy indicated neurological involvement. No symptoms of liver disease except coagulopathy were observed; however, steatofibrosis with cholestasis was found at autopsy. The girl died at the age of 2 months owing to the progressive general oedema, bleeding and cardio-respiratory insufficiency. Molecular analysis revealed two heterozygous mutations in the ALG8 gene: c.139A>C (p.T47P) and the novel mutation c.1090C>T (p.R364X). Conclusion: The prognosis of patients with ALG8 deficiency is unfavourable. The majority of affected children have early onset of the disease with heterogeneous symptoms including multiple organ dysfunction, coagulopathy and protein-losing enteropathy. Neurological impairment is not a general clinical symptom, but it has to be taken into consideration when thinking about ALG8 deficiency.

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***Title* of the article.**

# A new case of ALG8 deficiency (CDG-Ih)

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Congenital disorders of glycosylation (CDG) represent an expanding group of inherited diseases. One of them, ALG8 deficiency (CDG-Ih), leads to protein N-glycosylation defects caused by malfunction of glucosyltransferase 2 (Dol-P-Glc:Glc1-$Man_9$-$GlcNAc_2$-P-P-Dol glucosyltransferase) resulting in inefficient addition of the second glucose residue onto lipid-linked oligosaccharides. So far, only five patients have been described with ALG8 deficiency. We present a new patient with neonatal onset. The girl was born at 29$^{th}$ week of gestation complicated by oligohydramnion. Although the early postnatal adaptation was uneventful (APGAR score 8 and 9 at the 5$^{th}$ and 10$^{th}$ minutes), generalized oedema, multifocal myoclonic seizures, and bleeding due to combined coagulopathy were present since the first day. Diarrhea progressing to the protein-losing enteropathy with ascites and pericardial effusion developed in the 3$^{rd}$ week of life. Pharmacoresistant seizures and cortical, cerebellar and optic nerves atrophy indicated neurological involvement. No symptoms of liver disease except coagulopathy were observed, however steatofibrosis with cholestasis was found at autopsy. The girl died at the age of 2 months due to the progressive general oedema, bleeding and cardio-respiratory insufficiency. Molecular analysis revealed two heterozygous mutations in the *ALG8* gene: c.139A>C (p.T47P) and the novel mutation c.1090C>T (p.R364X). <u>*Conclusion:*</u> The prognosis of patients with ALG8 deficiency is unfavorable. Majority of affected children have early onset of the disease with heterogeneous symptoms including multi-organ dysfunction, coagulopathy and protein losing enteropathy. Neurological impairment is not a general clinical symptom, but it has to be taken into consideration when thinking about ALG8 deficiency.
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**A *concise 1 sentence take-home message* (synopsis) of the article, outlining what the reader learns from the article (this is usually printed on the (inside) back cover of *JIMD*).**

Clinical course of the disease in our patient further expands the awareness of symptoms diversity in patients with ALG8 deficiency, which includes multi-organ dysfunction with protein losing enteropathy, coagulopathy and central nervous system involvement.

**An *abbreviated title* for the running head.**

ALG8 deficiency

***References to electronic databases***
OMIM 608104

***A list of abbreviations.***

CDG - congenital disorder of glycosylation

Dol - dolichol

APTT - activated partial thromboplastin time

IEF – isoelectric focusing

PMM – phosphomannomutase

PMI – phosphomannose isomerase

LLO – lipid-linked oligosaccharide

NLO – protein N-linked oligosaccharide

CDT - carbohydrate-deficient transferrin

PLE – protein-losing enteropathy

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K. Vesela – molecular analysis, manuscript preparation
T. Honzik – clinical diagnostics, manuscript preparation
H. Hansikova – biochemical investigation
J. Semberova, Z. Stranak – attending physicians from neonatal intensive care unit
M.A. Haeuptle – biochemical investigation (LLO and NLO analysis)
T. Hennet – biochemical investigation (LLO and NLO analysis), supervisor
J. Zeman – clinical diagnostics, supervisor

***Name of one author who serves as guarantor*** **for the article**, accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

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The study was carried out in accordance with the Declaration of Helsinki of the World Medical Association and was approved by the Committee of Medical Ethics of the Faculty of Medicine and General Teaching Hospital in Prague. Informed consent was obtained from parents.

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## Introduction

Congenital disorders of glycosylation (CDG syndromes) comprise a large group of genetic diseases resulting from defects in the synthesis and the processing of glycans (Jaeken and Matthijs 2007). *ALG8* deficiency (CDG-Ih; OMIM 608104) is caused by pathological mutations in the *ALG8* gene (Chantret et al. 2003) coding for the enzyme glucosyltransferase 2 (Dol-P-Glc:Glc1-$Man_9$-$GlcNAc_2$-P-P-Dol glucosyltransferase). This enzyme is responsible for the addition of the second glucose residue onto the growing lipid-linked oligosaccharide (LLO) chain (Chantret et al. 2003). The *ALG8* gene is located on chromosome 11 and spans 38.7 kbp. Translation of its 1404 nucleotide-long open reading frame, organized in 13 exons results in a 467 amino acid long product.

So far, only five patients with ALG8 deficiency have been described (Charlwood et al. 1997; Chantret et al. 2003; Schollen et al. 2004; Eklund et al. 2005), presenting with dysmorphic features, hypotonia, oedema, coagulopathy, gastrointestinal disorders including protein-losing enteropathy (PLE), hepatomegaly and cardio-respiratory problems. Four patients had neonatal onset and deceased in early childhood. One patient (Chantret et al. 2003) manifested later at the age of 4 month and survived beyond 3 years of age.

The aim of our study was to present a detailed clinical picture of a new ALG8 deficient patient with neonatal onset to further expand the general knowledge about the clinical symptoms in this disorder.

## Case report

The girl was the first child of healthy, unrelated Caucasian parents. The pregnancy after *in vitro* fertilization was complicated with oligohydramnion progressing to anhydramnion. The child was born at $29^{th}$ week of gestation with a birth weight of 1420 g and length of 38 cm by acute caesarean section. Generalized oedema (anasarca) and mild hypotonia were observed since birth. Although, the early postnatal adaptation was feasible with APGAR score 8 and 9 at the $5^{th}$ and $10^{th}$ minutes, respectively, already during the first day of life multifocal myoclonic seizures and clinical signs of severe anemia developed. She was ventilated and inotropic treatment was initiated. Each sucking from endotracheal tube was complicated by mild bleeding episodes. Laboratory analyses at that time revealed pancytopenia (leucocytes $2.6.10^9$/l, controls >5.5; hemoglobin 85 g/l, controls >145; thrombocytopenia $61.10^9$/l, controls >150) and severe combined coagulopathy with very low levels of factor XI (2% in

comparison to controls), antithrombin III (20%) and protein C (2%). Activated partial thromboplastin time was markedly prolonged (APTT >180s). Almost no improvement of the coagulation status was seen after frequent administration of fresh frozen plasma, antithrombin III, protein C and factor VIIa concentrate. Hemorrhagic diathesis with bleeding necessitated repeated administration of packed red blood cells.

Although the clinical status seemed stabilized, since 3rd week of life progressive diarrhea, nasogastric tube feeding intolerance, abdominal distension followed on development of ascites and pericardial effusion were observed, as well as hypoalbuminemia (21.6 g/l, controls >35 g/l). However, the bleeding constantly dominated over tendency to thrombosis, thrombosis of vena cava inferior was documented at the age of one month. As a consequence of cardiac insufficiency and thrombotic episodes, liver congestion and portal hypertension developed leading to marked hepatosplenomegaly.

Oedema was permanent with progressive course. Even though we did not study alfa-1-antitrypsin clearance, the diagnosis of PLE was highly probable based on the fact that to maintain the normal concentration of albumin and immunoglobulins in blood necessitated practically continuous infusion of albumin (10% solution) and immunoglobulins substitution and on the fact that diarrhea persisted and losses of albumin into the urine was minimal.

The seizures were under control on phenobarbital treatment, but than generalized tonic seizures appeared at the age of 6 weeks. Brain ultrasound and eye investigations did not show any pathological findings till the age of 6 weeks, when mild cerebral cortical and atrophy and bilateral optic nerve atrophy was observed.

The girl was oxygen dependent on artificial lung ventilation or nasal CPAP until her death at the age of 2 months. The steatofibrosis with cholestasis found at the autopsy was striking, since no laboratory signs of liver failure or marked liver dysfunction were observed during the patients´ life. Brain post-mortem studies revealed mild cerebellar atrophy, but did not show any specific signs of cortical dysgenesis not even periventricular area changes typical for premature newborns.

The immediate cause of death was the progressive general oedema, bleeding and cardio-respiratory insufficiency leading to the multi-organ failure.

**Ethics**

The study was carried out in accordance with the Declaration of Helsinki of the World Medical Association and was approved by the Committee of Medical Ethics of the Faculty of

Medicine and General Teaching Hospital in Prague. Informed consent was obtained from the parents.

**Methods:**

***Biochemical methods:***

IEF of serum transferrin was carried out as described by van Eijk and van Noort (van Eijk and van Noort 1992). Iron-saturated serum was incubated overnight at 37˚C with neuraminidase from *Clostridium perfringens* (Boehringer), and subsequently separated by SDS- PAGE (5%T, 3%C; pH 3-10). PMM and PMI activities were measured in isolated lymphocytes according to the procedure described by Van Schaftingen and Jaeken (Van Schaftingen and Jaeken 1995).

LLO and NLO were prepared from control and patient fibroblasts as described previously (Haeuptle et al. 2008). Briefly, the oligosaccharides were metabolically labelled by incorporation of $^{3}$[H]-mannose. The LLO were extracted with organic solvents and hydrolysed by mild acid treatment. NLO were released from glycoproteins by incubation with PNGase F (New England BioLabs). Prior to HPLC analysis, the oligosaccharides were purified by ionic and hydrophobic chromatography.

***Molecular methods:***

All 13 exons of ALG8 gene amplified from patients gDNA were analyzed on an ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems). The c.139A>C mutation was confirmed by restriction fragment length polymorphism using the *MnlI* restriction endonuclease. For confirmation of the c.1090C>T mutation, we designed mismatch primers for the *HpaII* enzyme.

**Results:**

***Biochemical analyses:***

The possible diagnosis of CDG in this patient was confirmed by detection of hypoglycosylated transferrin isoforms by IEF. The analysis revealed an abnormal glycosylation pattern, with decreased tetrasialo- and increased disialo- and asialo- transferrin, which is typical for CDG type I. Neuraminidase treatment excluded the possibility of a transferrin polymorphism. PMM2 and PMI activities measured in isolated lymphocytes and cultivated fibroblasts were normal, thus excluding CDG-Ia and CDG-Ib.

***LLO analysis:***

In order to detect a possible defect in LLO assembly, skin fibroblasts isolated from the patient were labeled with $^{3}$[H]-mannose, and the glycans released from the lipid carrier were analyzed by HPLC. The LLO profile of the patient showed the accumulation of incomplete precursor structures corresponding to $GlcNAc_2Man_9$ and $GlcNAc_2Man_9Glc_1$ (Figure 1, left panel). This pattern is typical for ALG8 deficiency (CDG-Ih) (Chantret et al. 2003).

The accumulation of Dol-PP- $GlcNAc_2Man_9$- observed in the patient may be due to the deglucosylation of Dol-PP-$GlcNAc_2Man_9Glc_1$ by ER glucosidase II, that has arisen due to inefficient addition of the second glucose residue by the ALG8 glucosyltransferase.

***NLO analysis:***

The NLO analysis revealed an HPLC profile comparable to that obtained from healthy control subjects (Figure 1, right panel). This is in accordance with thin layer chromatography analysis of NLO in an earlier ALG8 deficient patient (Chantret et al. 2003).

***Molecular analyses:***

Two *ALG8* mutations in heterozygous form were detected in the patient. The first mutation (c.139A>C), already described in literature (Schollen et al. 2004), was combined with a novel mutation c.1090C>T. The index mutation, which is translated into the missense mutation p.T47P, was inherited from the father. The c.1090C>T mutation resulting in a premature stop codon (p.R364X) was found in heterozygous form in the mother, whereas it could not be found in 150 healthy controls.

**Discussion:**

The clinical course of the disease in our patient demonstrates, similar to the published cases (see Table 1), that the principal clinical and laboratory pathology is hemato-intestinal presentation. The majority of the patients were delivered prematurely and the first clinical symptoms, especially PLE, were presented during the first weeks of life. Only in one patient (surviving beyond three years of age) PLE and diarrhea developed later at the age of 4 months.

Anemia, pancytopenia, hypoalbuminemia and coagulopathy were observed in most patients. Coagulopathy in patients with ALG8 deficiency appears to be caused by a combination of hypoproduction of both, clotting factors and their inhibitors, and their increased consumption during chronic disseminated intravascular coagulation. The

coagulopathy in our patient seems to be more accentuated with profound bleeding in contrast to patients with other CDG syndromes. Difficult-to-treat hypoalbuminemia and coagulopathy resulted in the development of ascites, pericardial effusion, bleeding and early death.

Although craniofacial dysmorphy, inverted nipples and atypical fat pads were not present in our patient, dysmorphy was observed in three reported cases of ALG8 deficiency (Schollen et al. 2004; Eklund et al. 2005), whereas inverted nipples and fat pads were noticed in one case (Charlwood et al. 1997). Since osteopenia, cataracts (Eklund et al. 2005), lung hypoplasia (Schollen et al. 2004) and retinopathy (Chantret et al. 2003) were only found in a single child, we deduce that these symptoms cannot be assigned as typical for ALG8 deficient patients.

We did not observe elevated aminotransferases, but pronounced steatofibrosis with cholestasis was found at autopsy. Based on revision of clinical and laboratory reports from all six patients including our case, it seems that also mild tubulopathy and proteinuria belong to typical ALG8 deficiency symptoms. In addition, renal cortical and medullar microcysts were found in our patient.

Until the fifth case was published in 2005 by Eklund and colleagues (Eklund et al. 2005), ALG8 deficiency had been presented as a type of CDG without neurological manifestations (Schollen et al. 2004). Eklund et al. described a patient, who died at the age of 16 months and presented with psychomotor delay, hypotonia and leukoencephalopathy. In our case, we also observed marked hypotonia and multifocal myoclonic seizures since birth, bilateral optic nerve atrophy, cerebral cortical atrophy and mild cerebellar atrophy. On the other hand, the patient studied by Chantret and coworkers (Chantret et al. 2003) survived beyond three years (Eklund et al. 2005) and had no signs of any neurological impairment and strikingly, PLE and diarrhea subsequently disappeared. The mild clinical features of this patient could be explained by an elevated residual ALG8 expression. In summary, neurological impairment is not a general clinical symptom, but it has to be taken into consideration when thinking about ALG8 deficiency.

**Conclusion:**

We present a new patient with ALG8 deficiency (CDG-Ih), the sixth case described to date. The course of the disease in this new ALG8 case further expands the awareness of clinical symptoms diversity, which include multiorgan dysfunction with PLE, liver, kidney and central nervous system involvements and combined coagulopathy. Furthermore, we also identified a novel pathological mutation c.1090C>T (p.R364X) in the *ALG8* gene. The

diagnostics enabled genetic counseling and successful prenatal diagnostic in the affected family.

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